REMARKS

Applicant's attorney wishes to thank the Examiner for the careful consideration given to the present application. Currently, claims 1-8 and 19-20 are canceled and claims 9-18 and 21-40 are pending. Applicant addresses each of the rejections set forth in the Office Action in the order presented therein.

Related Applications

Applicant would like to again remind the Examiner of the related, co-pending U.S. Application No. 10/366,751 filed on February 14, 2003, which is presenting related claims (i.e. composition comprised of a suitable dosage amount of 1-methylpropyl-2-imidazolyl disulfide and a pharmaceutically acceptable carrier), which are currently under appeal.

Priority

Applicant notes that the Examiner has afforded the current application a priority date of December 12, 1997. This date is incorrect as the correct PCT filing date is December **5**, 1997. This priority document (i.e., U.S. Provisional Application No. 60/031,995) was filed on December 6, 1996. In fact, the inventors' recognition of "Thioredoxin as a Target for Cancer Chemotherapeutics" (the title of the provisional application filed on December 6, 1996) is the fundamental basis for the present application. While Applicant is not perfecting this claim to priority at this time, the Examiner should be aware that without recognizing thioredoxin as a target for chemotherapeutics, there would be no invention claimed herein.

35 U.S.C. § 102(a)- Powis

The Examiner has rejected claims 7-40 under 35 U.S.C. § 102(a) as being anticipated by Powis et al., Anti Cancer Drugs December 1996 7(3): 121-126. Applicant respectfully disagrees. As discussed in more detail below, it is respectfully submitted that Powis is not property citable as prior art under 35 U.S.C. § 102(a) as Powis is not "by another."

Applicant respectfully submits that Powis and Kirkpatrick are the co-inventors of the presently claimed subject matter, as the claims are currently directed to a drug comprising a 1-methylpropyl 2-imidazolyl disulfide and a pharmaceutically acceptable carrier for either injection or oral administration (e.g., claims 7-8 and 19-20) and further specify that 1-methylpropyl 2-imidazolyl disulfide irreversibly binds to thioredoxin, and in particular binds to a critical cysteine residue (i.e., Cys^{73}) of thioredoxin (e.g., claims 27-30). This is further supported

and established by the fact that in co-pending U.S. Application No. 10/366,751 a Declaration of Inventor dated September 9, 2005 of Kirkpatrick was submitted to the Office on October 10, 2005 stating that the subject matter being claimed in such application, including in particular claims to a composition comprised of a salt of 1-methylpropyl 2-imidazolyl disulfide and a pharmaceutically acceptable carrier, was solely invented by Kirkpatrick and that the other co-authors listed in Powis were not inventors of such subject matter. Similarly, in co-pending U.S. Application No. 10/366,751 a Disclaiming Declaration of Co-Author of Publication dated March 7, 2005 of Powis was submitted to the Office on April 7, 2005 stating that that the subject matter being claimed in *such* application, including in particular claims to a composition comprised of a salt of 1-methylpropyl 2-imidazolyl disulfide and a pharmaceutically acceptable carrier, was solely invented by Kirkpatrick and that the other co-authors listed in Powis were not inventors. Additionally, Powis is identified as the sole inventor of U.S. Patent No. 6,689,775 with claims directed to, e.g., methods of inhibiting tumor growth *in vivo* by contacting a tumor cell with a 2-imidazolyl disulfide, wherein the disulfide binds the cysteine at residue 73 of human thioredoxin. Accordingly and in light of the fact that the present claims are directed to both a drug comprising 1-methylpropyl 2-imidazolyl disulfide and a pharmaceutically acceptable carrier for either injection or oral administration and further specify that the disulfide irreversibly binds to thioredoxin, and in particular at the cysteine of residue 73 of thioredoxin, it is respectfully submitted that both Kirkpatrick and Powis are co-inventors on the present application and, as previously established through both the declarations and the issuance of the '775 Patent, such subject matter was invented by both Kirkpatrick and Powis, and the remaining co-authors on the Powis reference are not inventors of such subject matter and were only involved with respect to such an invention as an assistant to work under the supervision of the inventors and were listed solely to receive academic credit.

35 U.S.C. § 102(b)- Oblong

The Examiner has maintained the rejection of claims 7-30 under 35 U.S.C. § 102(b) as being anticipated by Oblong et al "Reversible inhibition of human thioredoxin reductase activity by cytotoxic alkyl 2-imidazolyl disulfide analogues," Cancer Chemother Pharmacology, 1994, 3-4: 434-438 as evidenced by Chaplan et al., (U.S. Patent No. 5,849,737) and Padmanaban (U.S. Application No. 2007/01059455). Applicant respectfully disagrees. Fundamentally and respectfully it is asserted that Oblong is not appropriate prior art in that

Oblong does not, among other things, teach "suitable dosage amount" as recited in the claims of the instant application. Moreover, Oblong fails to anticipate claims 7-30 because Oblong only teaches that the 2-imidazolyl disulfides of Oblong (including 1-methylpropyl 2-imidazolyl disulfide, also referred to as IV-2) inhibit the thioredoxin/thioredoxin reductase system (the "System"). As Applicant originally stated in the present application "[a]lthough these agents were originally identified as competitive inhibitors of thioredoxin reductase (Oblong JE, et al., Cancer Chemother. Pharmacol., 34:434-438(1994)…it has now been shown that they…bind irreversibly to Cys^{73} of thioredixon and…block its reduction by thioredoxin reductase." *See* priority PCT Application No. PCT/US97/22292 filed December 5, 1997 at page 33, lines 5-9. Oblong, in fact, naively attributes this inhibition to the inhibition of thioredoxin reductase ("TR") system (the entire thioredoxin:TR system being referenced as the "System") (see Abstract of Oblong). Oblong does not describe inhibition of thioredoxin (as determined and claimed by Applicant); and Oblong's inhibition of the System is not indicative of pharmaceutical effectiveness. Moreover, Oblong reports essentially identical Ki and IC_{50} values for III-2, IV-2 and VII-2, and further describe these compounds as inhibitors of the System. Applicant unexpectedly found that these select asymmetric disulfides being claimed herein behaved principally as inhibitors of thioredoxin rather than as substrates of TR (discussed in more detail below as it relates to the obviousness rejection.) Despite their similarities in structure and reported *in vitro* inhibitory activities, it was found that 1-methylpropyl-2-imidazolyl disulfide was, in fact, an inhibitor of thioredoxin, while structurally analogous compounds were functioning as substrates of TR. A difference of only <u>two</u> carbon atoms within the carbon chain (i.e. III-2 or VI-2 versus IV-2) renders a compound a substrate of TR instead of an inhibitor of thioredoxin, and dramatically impacts its suitability as a pharmaceutical composition. fact that 1-methylpropyl-2-imidazolyl disulfide is a suitable pharmaceutical composition and neither III-2 or VI-2 are effective was first taught by Applicant, and in itself is worth of protection.

Applicant is the first to disclose appropriate dosages of 2-imidazolyl disulfides, including 1-methylpropyl-2-imidazolyl disulfide, for injection and oral administration, and was the first to demonstrate the therapeutic effects of such dosages *in vivo*. For the foregoing reasons, Oblong fails to anticipate claims 7-40, and this rejection should be withdrawn.

35 U.S.C. § 103

The Examiner has rejected claims 31-40 under 35 U.S.C. § 103(a) as being unpatentable over Oblong as evidenced by Chaplan and Padmanaban. Applicant respectfully disagrees. The arguments presented above with regard to the rejection under 35 U.S.C. § 102(b) are incoporated herein by reference and will not be unnecessarily repeated.

(A) The art fails to teach or suggest a "suitable dosage amount" as recited in each of the independent claims

The claims all require a suitable dosage amount of 1-methylpropyl 2-imidazolyl disulfide. Oblong teaches that the disulfides of Oblong inhibit the thioredoxin/TR system. Oblong attributes this inhibition to the inhibition of TR (See Abstract of Oblong). Oblong does not describe inhibition of thioredoxin (as determined and claimed by Applicant); and Oblong's inhibition of the system is not indicative of pharmaceutical effectiveness. Oblong and Kirkpatrick report essentially identical Ki and IC_{50} values for III-2, IV-2 and VII-2, and further describe these compounds as inhibitors of thioredoxin/TR system. Applicant unexpectedly found that select asymmetric disulfides behaved principally as inhibitors of thioredoxin rather than as substrates of TR. Despite their similarities in structure and reported *in vitro* inhibitory activities, it was found that the presently claimed disulfide (i.e., 1-methylpropyl 2-imidazolyl disulfide) was, in fact, an inhibitor of thioredoxin, while structurally analogous compounds were functioning as substrates of TR (highlighted for the Examiner's convenience).

Table 2 of Applicant's co-pending U.S. Serial No. 10/366,751 is reproduced in its entirety below with highlight added to emphasize the importance and difference between the substrate, inhibitor, and the non-reactive nature of select asymmetric disulfides.

Table 2

Effects of 2-imidazolyl Disulfides On Human Thioredoxin Activity				
Compound	R	Type	Km (μM)	Ki
Ethyl 2-imidazolyl disulfide (VI-2)	$-CH_2CH_3$	Substrate	48.1	-
n-butyl 2-imidazolyl disulfide (III-2)	$-(CH_2)_3CH_3$	Substrate	43.1	-
1-methylpropyl 2-imidazolyl disulfide (IV-2)	$-CH(CH_3)CH_2CH_3$	Inhibitor	-	30.8
t-butyl 2-imidazolyl disulfide (IX-2)	$-C(CH_3)_3$	Non-reactive	-	-
benzyl 2-imidazolyl disulfide (DLK-36)	$-CH_2C_6H_5$	Inhibitor	-	30.9

As shown in Table 2, a difference of only two carbon atoms within the carbon chain (i.e. III-2 or VI-2 versus IV-2) renders the compound a substrate of TR instead of an inhibitor of thioredoxin, and dramatically impacts its suitability as a pharmaceutical composition, and may be determinative of whether it is an active compound (e.g., the Office's attention is directed to the inactivity of IX-2). While it may have been recognized in the art that all of these compounds may be of interest because they were disulfides, Applicant's discovery that structurally similar compounds behaved dramatically different is the very essence of determining the "suitable dosage amount" of the presently claimed compound. In fact, as it turned out, this difference in activity dictates where one or the other is suitable as a pharmaceutical composition at all. The fact that 1-methylpropyl 2-imidazolyl disulfide is a suitable pharmaceutical composition and neither n-butyl 2-imidazolyl disulfide (III-2) or ethyl 2-imidazolyl disulfide (VI-2) are effective is not yet appreciated. The fact that structurally similar compounds described in Oblong and Kirkpatrick behaved dramatically differently when later incorporated into a pharmaceutical composition for therapeutic use is also ignored by the Office. Applicant should not be penalized for their early disclosure of 1-methylpropyl 2-imidazolyl disulfide (i.e., Oblong) because they were simply reporting interesting activity of the compound as it related to the System. Until it was recognized that 1-methylpropyl 2-imidazolyl disulfide was an inhibitor of thioredoxin by irreversibly binding to thioredoxin, the suitability of asymmetric disulfides as pharmaceutically effective compounds was mere speculation.

Without knowing Applicant's disclosures that the claimed asymmetric disulfide was an inhibitor of thioredoxin, it is very possible that no amount of routine experimentation would result in a viable drug candidate. If IX-2 were chosen, for example, a suitable dosage amount may never have never been determined. Again, a seminal question is presented: why

would the skilled artisan choose IV-2 (an inhibitor), instead of IX-2 (a non-reactive species); or IV-2 instead of VI-2 (a substrate) without knowing their role as an inhibitor of thioredoxin, a substrate of thioredoxin reductase or a non-reactive species? Given the virtually identical Ki and other pharmacokinetic data presented in Oblong, a wrong choice would have resulted in the skilled artisan performing routine experimentation until the end of time and never finding an suitable dosage amount (or at least an suitable dosage amount that was not toxic).

(B) Applicant has rebutted any purported *prima facie* case of obviousness

(1) Applicant has established unexpected property of 1-methylpropyl 2-imidazolyl disulfide as an inhibitor of thioredoxin

Assuming arguendo that the Office has met its burden, Applicant has rebutted any such purported *prima facie* case of obviousness by establishing that Applicant found that the claimed disulfide (i.e., 1-methylpropyl 2-imidazolyl disulfide) was unexpectedly an inhibitor of thioredoxin, as opposed to the teaching in the art that asymmetric disulfides (including the presently claimed disulfide) were substrates of thioredoxin reductase (TR) (not inhibitors of thioredoxin).

In maintaining the rejection, it is respectfully asserted that the Examiner fails to appreciate the nature and importance of enzyme kinetics. Thioredoxin is a substrate of TR. In particular, TR reduces the active-site cysteine residue of oxidized thioredoxin (Trx) using NADPH as a co-factor. A simplified schematic of the intermediate interaction at any time between thioredoxin (Trx) in its oxidized and inactive form and thioredoxin reductase (TR) is presented below:

$$\text{Trx}_{\text{(inactive)}} \xrightleftharpoons{\text{TR}} \text{Trx}_{\text{(active)}}$$

Specifically, all of the available oxidized thioredoxin (i.e., inactive Trx) is reversibly reduced to the free sulhydryl form (i.e., active Trx).

Oblong describes that asymmetric disulfides (including PX-12) are competitive inhibitors of TR, and therefore are inhibitors of the system. Thus, Oblong at best teaches that asymmetric disulfides compete with Trx (thioredoxin) to be a substrate of TR. A simplified schematic of the reversible reaction at any time (T) between thioredoxin and the asymmetric disulfides (AD), both as competitive inhibitors, as described by Oblong is presented below:

TR

$$Trx_{(inactive)} + AD \rightleftharpoons Trx_{(active)} + AD_{(reduced)} + Trx_{(inactive)}$$

According to Oblong, assuming catalytic amounts amounts of TR, some thioredoxin remains inactive because the asymmetric disulfide competes with endogenous thioredoxin as a substrate for TR. Oblong reports essentially identical Ki and IC_{50} values for III-2, IV-2 (i.e., 1-methylpropyl 2-imidazolyl disulfide) and VII-2 (See Table 1 and abstract of Oblong). Based upon the teachings of Oblong, one skilled the art would expect that 1-methylpropyl 2-imidazolyl disulfide would be a substrate of TR. One skilled in the art would also expect that 1-methylpropyl 2-imidazolyl disulfide and the other asymmetric disulfides would be general, non-specific inhibitors of any endogenous system involving disulfide exchange mechanism based upon the teachings of the prior art that asymmetric disulfides inhibited glutathione reductase, which also involves a disulfide exchange mechanism.

1-methylpropyl 2-imidazolyl disulfide was unexpectedly a direct inhibitor of thioredoxin because it irreversibly bound to thioredoxin and prevented thioredoxin from being a viable substrate for TR. A simplified schematic of the reaction between thioredoxin and 1-methylpropyl 2-imidazolyl disulfide (also referred to as PX12) as discovered by Applicant is presented below:

$$Trx_{(active)} + PX12 \longrightarrow [Trx\text{-}PX12_{(inactive;\ irreversible)}] \not\rightarrow TR$$

This essentially irreversible binding between thioredoxin and 1-methylpropyl 2-imidazolyl disulfide (PX12) removes the thioredoxin as a candidate for further processing by TR. As disclosed in the present application, it has been theorized by Applicant that the branching of the alkyl moieties of the 1-methylpropyl 2-imidazolyl disulfide and the benzyl group of DLK-36 prevent reduction by TR, and therefore they were not suitable substrates of TR. This allows them to irreversibly bind to thioredoxin (Trx). Importantly as an inhibitor of thioredoxin (not a substrate of TR), once bound to thioredoxin, these agents prevent the modified thioredoxin reduction by TR. Accordingly, the presently claimed agents act principally as irreversible inhibitors of thioredoxin.

Unequivocally, Applicant's unexpected discovery that 1-methylpropyl 2-imidazolyl disulfide (PX12) is an irreversible inhibitor of the endogenous substrate (i.e. thioredoxin) is vastly different from the expected function of being a competitive inhibitor with the endogenous substrate. One ordinarily skilled in the art would expect a competitive inhibitor

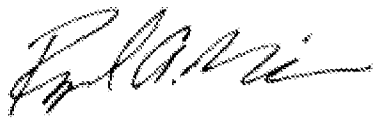
(substrate of) of a disulfide-based enzyme (e.g. TR) to also be a substrate for disulfide-based enzymes. One skilled in the art understands that the effectiveness as a competitor and the off-target competition with other substrates would negatively impact the viability of using such asymmetric disulfides in a pharmaceutical composition. In contrast, Applicant discovered that 1-methylpropyl 2-imidazolyl disulfide (PX12) and other select asymmetric disulfides (e.g., DLK-36) were unexpectedly found to directly inhibit thioredoxin by irreversibly binding to thioredoxin. In fact, Applicant reported that 1-methylpropyl 2-imidazolyl disulfide and DLK-36 were not inhibitors of glutathione reductase, as expected. The implications of Applicant's unexpected discovery that 1-methylpropyl 2-imidazolyl disulfide and DLK-36 were specific, irreversible inhibitors of thioredoxin is that these compounds are suitable for use as a therapeutic or pharmaceutical composition because they demonstrate (i) specificity for inhibiting thioredoxin, in contrast to the non-specificity of the other asymmetric disulfides and (ii) increased efficacy for inhibiting thioredoxin associated cellular proliferation. The specificity decreases the likelihood of off-target effects (i.e., inhibition of other redox systems such as glutathione reductase) when administered to a subject. It should be noted that the irreversible inhibition of thioredoxin also increases the efficacy of the compound as an inhibitor of cellular proliferation.

CONCLUSION

Applicant has timely filed this response with the payment of a three-month extension fee. In the event that an additional fee is required for this response, the Commissioner is hereby authorized to charge such fees to Deposit Account No. 50-0436.

Should the Examiner have any questions or comments, or need any additional information from Applicant's attorney, he is invited to contact the undersigned at his convenience.

Respectfully submitted,



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